Periodontal Infection as a Possible Risk Factor for Preterm Low Birth Weight

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PERIODONTAL DISEASES ARE GRAM-NEGATIVE ANAEROBIC INFECTIONS that can occur in women of childbearing age (18 to 34 years). In the present investigation we sought to determine whether the prevalence of maternal periodontal infection could be associated with preterm low birth weight (PLBW), controlling for known risk factors and potential covariates. A case-control study of 124 pregnant or postpartum mothers was performed. PLBW cases were defined as a mother with a birth of less than 2,500 g and one or more of the following: gestational age <37 weeks, preterm labor (PTL), or premature rupture of membranes (PROM). Controls were normal birth weight infants (NBW). Assessments included a broad range of known obstetric risk factors, such as tobacco use, drug use, alcohol consumption, level of prenatal care, parity, genitourinary infections, and nutrition. Each subject received a periodontal examination to determine clinical attachment level. PLBW cases and primiparous PLBW cases (n = 93) had significantly worse periodontal disease than the respective NBW controls. Multivariate logistic regression models, controlling for other risk factors and covariates, demonstrated that periodontal disease is a statistically significant risk factor for PLBW with adjusted odds ratios of 7.9 and 7.5 for all PLBW cases and primiparous PLBW cases, respectively. These data indicate that periodontal diseases represent a previously unrecognized and clinically significant risk factor for preterm low birth weight as a consequence of either PTL or preterm PROM. *J Periodontol 1996;67: 1103–1113.*

Key Words: Infant, low birth weight; periodontal diseases/adverse effects; pregnancy; risk factors; infant, premature.

Preterm infants who are born with low birth weights (LBW, i.e. <2,500 g) represent a major social and economic public health problem, even in industrialized nations. Although there has been an overall decline in infant mortality in the United States over the past 40 years, preterm LBW remains a significant cause of perinatal mortality and morbidity. A 47% decrease in the infant mortality rate to a level of 13.1 per 1,000 live births occurred between 1965 and 1980,[1] but that rate has not significantly improved over the last decade. Most of the evidence indicates that the most recent decline in infant mortality largely can be attributed to increased survival of low birth weight infants, as a result of more intensive hospital-based management of LBW infants.[2,3] While there has been a decrease in infant mortality over the last 40 years, there has been minimal decline in the incidence of LBW. In the U.S., approximately one in 10 deliveries results in a preterm LBW (PLBW) infant, usually as a direct consequence of preterm labor (PTL) or premature rupture of membranes (PROM).[4] More than 60% of the mortality that occurs among infants without anatomic or chromosomal congenital defects is attributable to preterm LBW.[2] These infants account for 5 million neonatal intensive care unit hospital days per year at an annual cost of greater than $5 billion.[5] The overall cost to society in terms of suffering and long-term disabilities, however, far exceeds these monetary estimates. Most long-term disability cases, for example, begin as low birth weight infants. Thus, the emotional, psychological, and financial burdens on families who experience PLBW can have profound and long-term consequences on society. To address this problem, some feel more emphasis should be placed on prevention rather than costly tertiary care.[6] McCormick[2] states, "the decline in neonatal mortality will be sustained only through the prevention of low-weight births and increased attention to the efficacy of services

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in the antenatal period." Some risk factors that have been associated with PLBW include: high (>34 years) or low (<17 years) maternal age, African-American race, low socioeconomic status, inadequate prenatal care, drug abuse, alcohol and tobacco use, hypertension, genitourinary tract infections, diabetes, and multiple pregnancies.[4,7] However, there is still disagreement as to the magnitude of these various factors' effect on birth outcome. Recent reports have evaluated the efficacy of antenatal maternal care programs that allow increased emphasis on the prevention of controllable risk factors. Data from these studies indicate that, in spite of substantial increases in the proportion of women beginning prenatal care in the first trimester, there still has been a relatively small decrease in the proportion of LBW.[8,9] As summarized in a recent review by Gibbs et al.,[6] the principal reason cited for the continued high LBW rate is a "poor understanding of antenatal factors that contribute to an infant with LBW." Thus, despite our increased understanding of the risk factors that contribute to PLBW, there remains a considerable lack of sensitivity in predictive models which can serve as a framework for intervention strategies. Even when considering all the "traditional" risk factors—smoking, genetics, alcohol, prenatal care, nutrition, urinary tract infection, and others—about 25% of PLBW cases occur without even a candidate or suspected risk factor. Thus, many groups including the National Academy of Sciences have suggested continued research into the causes and management of preterm labor and other obstetric complications leading to LBW.[5]

Several studies have demonstrated an association between infection and PLBW. The first evidence of this association involved the increased prevalence of maternal lower genitourinary tract infections with pregnancy complications such as PTL and LBW.[10,11] Other investigators have looked at the effects of subclinical urinary tract infections on pregnancy outcome. Rates of preterm delivery 1.5 to 2.3 times normal have been found among women with a symptomatic group B streptococci bacteriuria.[12,13] Gram-negative *Bacteroides* species have also been investigated as to their association with preterm delivery and/or premature rupture of membrane. One study showed a 40% increase in preterm delivery rates in mothers who were colonized with cervical *Bacteroides* at their initial prenatal visit.[14] Vaginal *Bacteroides* colonization was associated with a 60% increased risk of preterm delivery in a recent study.[15] Inflammation of the extraplacental membrane (chorioamnionitis) has been detected in up to four times as many mothers with preterm deliveries as in those with normal term deliveries.[16] Although there is a strong relationship between chorioamnionitis and placental infection, 18% to 49% of placentas with histological evidence of chorioamnionitis have negative cultures.[17] Thus, evidence exists that inflammation in the fetal-placental unit can be present without any sign of bacterial infection. Furthermore, genitourinary tract infections can be associated with LBW, without infection of the fetal-placental unit.[17] These observations have supported the widely held, current opinion that preterm LBW that occurs as a result of infection is mediated indirectly, principally by the translocation of bacterial products such as endotoxin (lipopolysaccharide, LPS) and by the action of maternally produced inflammatory mediators.[6]

Several scientists investigating the molecular basis of preterm LBW (see Gibbs et al.[6]) point to common cellular and biochemical pathways that seem to mediate the pathogenesis of PLBW, irrespective of the associated risk variables. For example, intra-amniotic levels of PGE_2 and TNF-α rise steadily throughout pregnancy until a critical threshold level is reached to induce labor, cervical dilation, and delivery. Since these molecules appear to be normal physiological mediators of parturition, it is not surprising that genitourinary tract infections, which result in the excessive secretion of these mediators by the decidua and trophoblastic cell layers, result in preterm delivery and low birth weight. However, the observation of elevated PGE_2 and TNF-α as a consistent and reproducible feature of PLBW, even in the absence of any clinical or subclinical genitourinary tract infections, has prompted Romero and others[5,18,19] to the conclusion that most PLBW cases are "probably caused by an infection of unknown origin." It is our hypothesis that periodontal infections, which serve as reservoirs for Gram-negative anaerobic organisms, lipopolysaccharide (LPS, endotoxin), and inflammatory mediators including PGE_2 and TNF-α, may pose a potential threat to the fetal-placental unit. This concept is supported by recent experiments in the pregnant hamster model. In this model, localized, non-disseminating subcutaneous infections with *Porphyromonas gingivalis* (a common periodontal pathogen) can significantly reduce fetal weight by up to 25%.[20] This infection is associated with increases in PGE_2 and TNF-α which appear to determine the magnitude of the growth retardation response.[20] Furthermore, experimental periodontitis in the pregnant hamster can retard fetal growth.[21] These observations have prompted us to investigate a potential association between periodontal disease and preterm LBW in humans. In the present investigation, we report that severe periodontal disease in the mother is a previously unrecognized risk factor for preterm LBW.

MATERIALS AND METHODS

Patient Selection

We requested participation from women while they were being seen for routine care in the Prenatal Care Clinic at the University of North Carolina Hospitals or within 3 days postpartum. A total of 132 volunteer mothers were entered into the study. Eight were excluded from the clinical portion of the study due to either current genitouri-

nary tract infection with concurrent antibiotic therapy, or risk factors for bacterial endocarditis, which would require prophylactic antibiotics for periodontal examination. The total study sample was 124 mothers. This study was approved by the Institutional Review Board for the protection of human subjects.

All subjects selected were registered patients of the UNC Prenatal Care Clinic. Twelve percent of the subjects were recruited for participation during clinic visits and the remainder from the hospital. Data were pooled from these two sites since no site sampling bias was observed by a systematic review of the obstetric history profile, biographical data, and current pregnancy data. The Prenatal Care Clinic population (1991 to 1992) included 871 births of which 8.5% were preterm premature rupture of membranes (PROM) or preterm labor-associated low birth weights (i.e. < 2,500 g). Thirty-three percent of the total prenatal care population was black and 67% white. Within the PROM or preterm labor low birth weight mothers, 45% were black and 55% white; within the normal birth weight term deliveries, 33% were black and 67% were white.

Pregnancy Outcomes and Risk Factors

Information on current and historical pregnancy outcome was obtained from two sources. Detailed data about previous pregnancies and the outcome of the current pregnancy were gathered from the patient's prenatal record and history from current and previous pregnancies. A mother was considered to have a positive preterm LBW history if, during the current pregnancy or previous pregnancies, she had an infant with a birth weight <2,500 g with any of the following outcomes: spontaneous (or non-elective) abortion prior to 12 weeks after gestation, preterm labor (defined as contractions and cervical change necessitating medical intervention), preterm rupture of the membranes requiring delivery at less than 36 completed weeks gestational age, or birth with gestational age less than 36 completed weeks. Gestational age was verified by ultrasound examination. In women not receiving prenatal care after 24 weeks gestation history, sequential ultrasound examination and post-natal examination were used to estimate the gestational age, which was re-evaluated after delivery with Dubowitz examination. Information was collected on known risk factors and included the following: previous pregnancy history—number carried to full-term, number with preterm delivery prior to 37 weeks, number of previous pregnancies aborted, number of spontaneous abortions, number with live births; current pregnancy—maternal age at delivery, birth weight, and gestational age. In addition, the following variables were scored as either present or absent: intrauterine growth retardation, fetal death, fetal abnormality, twin delivery, placentae abruptio, placental previa, uterine atony, uterine fibroids and Rh factor isoimmunity, severe or mild preeclampsia. Maternal race was listed as white, black, Hispanic, Asian, American Indian, or other. Onset of prenatal care was categorized as beginning routine care prior to 20 weeks' gestational age, between 20 and 25 weeks' gestational age, after 25 weeks, or no routine care. The adequacy of prenatal care also was assessed as adequate or inadequate, being <six prenatal visits, and domestic violence was screened using the McFarland assessment. Tobacco use was recorded as none, less than one pack per day, or more than one pack per day. The use of alcohol was recorded as none, less than six drinks per week, or more than six drinks per week. Illicit drug use was recorded. Any patient with a hematocrit <28% or 9 g was scored as anemic, and any patient with less than 20 pounds weight gain was considered as low maternal weight gain. Other outcome variables included gestational diabetes, hypertension with or without proteinuria or polynephritis. Other risk factors assessed included bacteriuria, cervicitis, bacterial vaginosis, vaginitis, amnionitis, dysplasia, *Chlamydia* infection, *Gardnerella* infection, Group B streptococcus colonization, *Trichomonas* infection, HIV, *Neisseria* gonorrheal, Herpes simplex infection, syphilis, or *Condyloma acuminata.*

Measurement of Periodontal Status

A full-mouth periodontal exam was performed on all 124 mothers either at the time of volunteering at the UNC Memorial Hospitals (UNCMH) prenatal clinic or at the UNC School of Dentistry Clinical Research Center within 3 days of their delivery at UNCMH. Full-mouth data were recorded on clinical attachment levels (CAL) and probing depths (PD) on six sites per tooth. CAL was measured with a manual probe (UNC-15), using the cementoenamel junction as a reference point. Bleeding on probing also was measured and expressed as the percentage of sites exhibiting this response. Examiners were masked from obstetric data.

Case Definitions

Our first definition of a case and a control was based on the current birth. However, we found that a history of a previous PLBW birth was strongly associated with clinical attachment level and was a very strong confounder. Although CAL is the most commonly used measure of periodontal disease, it is an accretion measure and can include both active and inactive disease sites. This is because individual sites with periodontal disease exhibit periods of activity and quiescence, and attachment gain is a much less frequently occurring phenomenon than attachment loss. Thus, once attachment loss occurs, the evidence of disease remains in the mouth and that site may or may not be currently active. This means that our measures of clinical attachment level also may have occurred with a previous PLBW infant, but that the current birth was NBW. Thus, we decided to deal with this time se-

quence problem by creating two definitions of a case. The first definition of a case was mothers with a current or previous birth in which the infant was PTL or PROM with PLBW <2,500 g. Controls were mothers with all birth weight infants ≥2,500 g without history of PTL or PROM. The second definition of a case was the same as for case and control, except that the group was restricted to mothers having their first baby (primiparous).

Data Analyses

Pregnancy outcomes were categorized into cases and controls for 2 groups of mothers—all mothers and primiparous mothers. Controls were defined as mothers with one or more full-term, birth weight infants ≥2,500 g without history of PTL or PROM, and cases were mothers with one or more PTL or PROM with PLBW <2,500 g. For each group, periodontal disease status was first defined using full-mouth, mean clinical attachment levels (mean CAL mm/site) for each patient, pooling to form group means.

To adjust the association between periodontal disease measures and case-control status for other risk factors and confounders, multivariate logistic regression methods were used following methods described in detail by Beck.[22] Two multivariate logistic regression models were developed using a dichotomized outcome as having had a low birth weight infant this pregnancy or a previous pregnancy (cases) or having had all normal birth weight infants (controls). A second case-control definition was used for modeling to limit the data to primiparous women to remove any effects of previous births or any potential error in previous partus reporting. Thus, additional models were developed using a case definition of primiPLBW, with primiNBW mothers serving as controls. Periodontal status was operationalized both as mean attachment level and as extent of attachment level using a 2, 3, or 4 mm threshold (i.e., the percentage of sites with clinical attachment level ≥ 2, 3, or 4 mm, respectively). These are referred to as Extent N scores, where N = CAL threshold. Severity scores are the mean CAL in sites with CAL ≥ N as defined by the extent score.[23] For example, severity 2 is the mean CAL in sites with CAL ≥ 2 mm. Relationships between all exposure and outcome variables were explored with bivariate analyses. Then, relationships between primary risk factors (including periodontal status) and low birth weight were assessed using odds ratios and 95% confidence limits both as crude odds ratios and stratified by race, age, and number of previous births. These analyses indicated that for many PLBW cases, the extent of attachment loss ≥ 3 mm (Extent 3 score) was strongly associated with birth weight. Extent scores less than 2 would be consistent with periodontal health or gingivitis, with extent scores greater than 3 reflecting periodontitis-associated attachment loss. Although higher extent or severity scores increased the crude odds ratio, there was a greater variability and less confidence in the magnitude of odds ratio. For this reason we used a more conservative estimate of disease extent (the Extent 3 variable). This continuous variable was dichotomized at 60%, meaning that people with attachment loss of 3+ mm affecting less than 60% of their sites were more likely to be in the control group. This extent variable is labeled as Extent 3:60. Finally, a logistic regression model was developed with case vs. control status as the outcome and Extent 3:60 being the periodontal status exposure of interest. After the crude relationship between periodontal status and birth weight was determined, control variables and other risk factors were tested in the model. After the final main effects model was established, interaction effects were allowed to enter the model in a stepwise fashion. A similar method was used to develop logistic regression models using the primiparous subset of patients. For the primiparous cases, mean CAL, Extent 3 and severity 3 scores were all significantly associated with PLBW case status, as compared to primiparous controls. As before, final logistic regression models were developed with primiparous cases vs. primiparous controls as the outcome and Extent 3:60 being the periodontal status exposure variable.

RESULTS

The demographics and other characteristics of cases and controls, as well as the primiPLBW cases and primiNBW controls, appear in Table 1. The mean age for cases was 25 ± 6.3 (SD) years, which was not significantly different from controls at 22 ± 3.4 years. The primiparous mothers tended to be younger with primiparous cases having a mean age of 23.6 ± 6.5 and primiparous controls 21.7 ± 3.6. However, age distribution was not significantly different comparing cases to controls for the dataset or the primiparous subset. Similarly, there was not a significant difference in the racial distribution of cases vs. controls with blacks representing slightly over half the population studied. The parity was not significantly different between cases and controls, with the primiparous mothers having no previous live births by definition. Neither tobacco use, alcohol use, nor level of prenatal care differed among the groups. Overall, 76.6% of the mothers abstained from tobacco use, 89.5% used no alcohol, and most mothers (79.0%) received prenatal care beginning prior to 20 weeks gestation. A positive history of cystitis that was not concurrent with pregnancy was found more than twice as often in controls as compared to cases (36% vs. 15%). These affected mothers were all treated with systemic antibiotics. This obstetric history variable was the only marginally statistically significant risk factor for this population (i.e., $P > 0.20$), and it appeared to have a slight protective effect in that it decreased the odds of a PROM or PTL-associated LBW. No other obstetric risk variables demonstrated a statistically significant association with

Table 1. Demographics and Descriptions of Cases and Controls

Variable	Any PLBW Cases (n = 93)	All NBW Controls (n = 31)	PrimiPLBW Cases (n = 46)	PrimiNBW Controls (n = 20)
Age (years)				
14–19	25 (27%)	7 (23%)	22 (48%)	8 (40%)
20–24	18 (19%)	14 (45%)	17 (37%)	12 (60%)
25–29	29 (31%)	10 (32%)	7 (15%)	0 (0%)
30–34	14 (15%)	0 (0%)	0 (0%)	0 (0%)
35–40	7 (8%)	0 (0%)	0 (0%)	0 (0%)
Race				
Black	54 (58%)	19 (61%)	24 (52%)	13 (65%)
White	28 (30%)	9 (29%)	17 (37%)	6 (30%)
Hispanic	8 (9%)	3 (10%)	3 (6%)	1 (5%)
Asian	3 (3%)	0 (0%)	2 (4%)	0 (0%)
Number of previous live births				
0	46 (50%)	20 (65%)	46 (100%)	20 (100%)
1	26 (28%)	8 (25%)	—	—
2	12 (13%)	3 (10%)	—	—
3	4 (4%)	0 (0%)	—	—
4	3 (3%)	0 (0%)	—	—
5+	2 (1%)	0 (0%)	—	—
Tobacco use				
Never	72 (77%)	23 (74%)	37 (80%)	14 (70%)
<1 pack/day	17 (18%)	8 (26%)	7 (15%)	6 (30%)
>1 pack/day	4 (4%)	0 (0%)	2 (4%)	0 (0%)
Alcohol use				
None	87 (93%)	27 (87%)	44 (96%)	18 (90%)
<6 drinks/week	5 (5%)	3 (10%)	2 (4%)	1 (5%)
>6 drinks/week	1 (1%)	1 (3%)	0 (0%)	1 (5%)
Prenatal care				
None	10 (11%)	1 (3%)	3 (7%)	0 (0%)
Began at 30 weeks	1 (1%)	NA	0 (0%)	0 (0%)
Began at 25 weeks	3 (3%)	1 (3%)	1 (2%)	2 (10%)
Began at 20 weeks	8 (9%)	NA	5 (11%)	0 (0%)
Before 20 weeks	71 (76%)	27 (87%)	37 (80%)	18 (90%)
History of bacteriuria				
Yes	14 (15%)	11 (36%)	3 (7%)	8 (40%)
No	79 (85%)	20 (64%)	3 (7%)	12 (60%)

– Data not available.

PLBW in this relatively small study population. In contrast, the periodontal disease indicators showed significant differences between cases and controls.

Although mean CAL measures are generally insensitive measures of disease, a comparison of the mean CAL between case and control mothers demonstrated significant differences. Figure 1 illustrates that the 93 PLBW cases had a mean CAL of 3.10 ± 0.74 mm/site. By comparison, the 31 NBW controls had a mean CAL of 2.80 ± 0.61 mm/site, which was significantly less disease than the cases at $P = 0.04$. The magnitude of the difference in disease severity is even more marked when comparing the primiPLBW cases to the primi controls. The mean CAL for primi cases was 2.98 ± 0.84 mm/site vs. 2.56 ± 0.54 mm/site for primi controls. Thus, the primiparous cases had significantly more severe periodontal disease at $P = 0.03$.

Table 2 shows the case-control comparisons using other periodontal disease indicators including mean probing depth, extent, and severity scores. The Extent 3 score (i.e., percentage of sites with 3+ mm CAL) demonstrated sig-

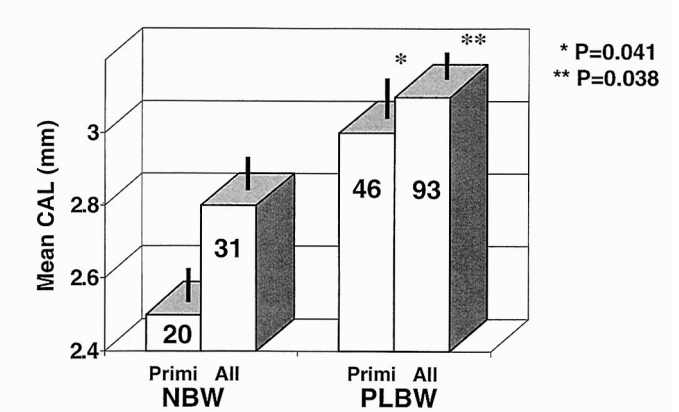

Figure 1. Mean clinical attachment loss in cases vs. controls.

Table 2. Periodontal Disease Indicators

Variable	Any PLBW Cases Mean ± SD	All NBW Controls Mean ± SD	PrimiPLBW Cases Mean ± SD	PrimiNBW Controls Mean ± SD
Probing depth (mm/site)	3.17 ± 0.68	2.99 ± 0.43	3.12 ± 0.79	2.87 ± 0.40
Clinical attachment level (mm/site)	3.10 ± 0.74*	2.80 ± 0.61	2.98 ± 0.84†	2.56 ± 0.54
Extent *N* scores (proportion of sites)				
Extent 2	0.94 ± 0.08	0.91 ± 0.11	0.93 ± 0.08	0.88 ± 0.13
Extent 3	0.69 ± 0.19‡	0.59 ± 0.25	0.65 ± 0.18§	0.49 ± 0.23
Extent 4	0.31 ± 0.21	0.24 ± 0.21	0.27 ± 0.19	0.17 ± 0.17
Severity *N* scores (mm/site)				
Severity 2	3.20 ± 0.67	3.00 ± 0.51	3.14 ± 0.77‖	2.7 ± 0.42
Severity 3	3.60 ± 0.57	3.50 ± 0.34	3.57 ± 0.70	3.33 ± 0.20
Severity 4	4.30 ± 0.49	4.20 ± 0.21	4.34 ± 0.64	4.13 ± 1.23

*$P = 0.038$.
†$P = 0.041$.
‡$P = 0.021$.
§$P = 0.007$.
‖$P = 0.016$.

nificant case-control differences at $P = 0.02$. Although not statistically significant, other indicators of disease such as PD and severity scores demonstrated a consistent trend for cases having worse periodontal disease than controls. Cases tended to have higher extent scores, indicating that the disease was more generalized, affecting more sites around the teeth, as opposed to having a few severely involved teeth. The primiparous cases had significantly more severe periodontal disease than primiparous controls, based upon mean CAL ($P = 0.03$), Extent 3 scores ($P = 0.004$), and severity 2 scores ($P = 0.016$). Thus, the periodontal condition of the primiparous cases was significantly worse clinically, as well as statistically, using several different disease indicators as compared to primiparous controls.

The unadjusted odds ratio and 95% confidence limits for several potential risk factors for PLBW appear in Table 3A. As stated before, age, race, parturition, tobacco use, alcohol use, and level of prenatal care do not appear to influence the observed frequency of PLBW in this dataset. Only a previous history of cystitis and Extent 3 scores are significantly associated with PLBW. As mentioned before, a positive history of bacteriuria was associated with a 3-fold decrease in the risk for PLBW at $P = 0.028$. Periodontal disease, which was a sufficient infectious burden to elicit at least 3 mm of CAL at a minimum of 60% of the sites, was associated with a crude odds ratio of 5.9 at $P = 0.0025$. Thus, without controlling for any other variables in the model, periodontal disease increased the risk for PLBW almost 6-fold. This same trend was observed in the primiparous mothers, as shown in Table 3B. A history of cystitis had a 10-fold decrease in odds ratio at $P = 0.002$, and the Extent 3:60 score was associated with a crude odds ratio of 6.7 at $P = 0.0034$ by Fisher exact. However, these odds ratios do not consider the effect of other risk factors or confounders. The final full logistic models appear in Table 4.

In creating the model, we decided to use race, age, and parity as control variables because they appeared to affect the relationship between other risk factors and birth weight and there were more black cases than in the UNC Prenatal Care Clinic population. Race and previous births were not significant but were left in the model. Age of the mother was significant, indicating there was a small increase in the odds of being in the low birth weight group with each increasing year of maternal age. A large number of other risk factors for low birth weight were tested in the model. The presence of bacterial vaginosis for the current pregnancy was the only other risk factor that was significant, indicating that those women who had a current infection were at lower risk of having low birth weight infants. We interpret this unexpected relationship as being due to treatment with antibiotics for that infection during the pregnancy. These patients were treated with metronidazole, which may have reduced the periodontal microbial burden. Use of alcohol was not significant in the model, but we felt it was important to leave it in the model because the odds ratio strongly indicated that non-use of alcohol was protective and that there were very few women who used alcohol in the study. Notably absent from the model were variables representing the lack of prenatal care and smoking as risk factors. This occurred because few of the subjects had these characteristics.

It is evident that after adjusting for all the other factors in the model, mothers with periodontal disease have more than seven times the odds of being cases. It also is impressive that when comparing the adjusted odds ratio (7.5) with the crude odds ratio (5.9), we find that the presence of the other variables in the model enhance the

Table 3A. Unadjusted (Crude) Odds Ratios for PLBW Risk Factors Using All Cases and Controls

Variable	Any PLBW Cases (n = 93)	All NBW Controls (n = 31)	Crude Odds Ratio	95% Confidence Limit
Age				
Mean ± SD	25 ± 6.3	22 ± 3.4	1.1	1.0, 1.2
Race				
Black	54 (58%)	19 (61%)	0.9	0.4, 2.0
Non-black	39 (42%)	12 (39%)		
Number of live births				
1	46 (59%)	20 (64%)	1.9	0.8, 4.3
2+	47 (51%)	11 (36%)		
Tobacco use				
Ever	21 (23%)	8 (26%)	0.8	0.3, 2.2
Never	72 (77%)	23 (74%)		
Alcohol use				
Yes	6 (7%)	4 (13%)	0.5	0.2, 1.8
No	87 (93%)	27 (87%)		
Prenatal care				
None	10 (11%)	1 (3%)	3.6	0.4, 29.4
Any	83 (89%)	30 (97%)		
History of bacteriuria				
Yes	14 (15%)	11 (35%)	0.3*	0.1, 0.8
No	79 (85%)	20 (65%)		
Extent 3:60				
Yes	87 (94%)	22 (71%)	5.9†	1.9, 18.4
No	6 (6%)	9 (29%)		

*P = 0.028.
†P = 0.0025.

Table 3B. Unadjusted (Crude) Odds Ratios for PLBW Risk Factors Using Primiparous Cases and Controls

Variable	PrimiPLBW Cases (n = 46)	PrimiNBW Controls (n = 20)	Crude Odds Ratio	95% Confidence Limit
Age (continuous)	23.6 ± 6.5	21.7 ± 3.6	1.1	(0.96, 1.2)
Race				
Black	24 (52%)	13 (65%)	0.6	(0.17, 1.95)
Non-black	22 (48%)	7 (35%)		
Tobacco use				
Ever	9 (20%)	6 (30%)	0.6	(0.20, 2.2)
Never	37 (80%)	14 (70%)		
Alcohol use				
Yes	2 (4%)	2 (10%)	0.4	(0.03, 6.14)
No	44 (96%)	18 (90%)		
Prenatal care				
None	3 (7%)	0 (0%)	0.7	(0.20, 37.1)
Any	43 (93%)	20 (100%)		
History of bacteriuria				
Yes	3 (7%)	8 (40%)	0.1*	(0.02, 0.54)
No	43 (93%)	12 (60%)		
Extent 3:60				
Yes	41 (89%)	11 (55%)	6.7†	(1.6, 30.0)
No	5 (11%)	9 (45%)		

*P = 0.002.
†P = 0.0034.

Table 4. Multivariate Logistic Regression Model for PLBW Using All Cases and Controls

Variable	Parameter Estimate	Standard Error	Probability Chi-Square	Odds Ratio	95% Confidence Levels
Extent 3:60	2.0152	0.6857	0.0033	7.5	(1.95, 28.8)
Race = non-black	−0.3925	0.4881	0.4214	0.7	(0.26, 1.76)
Age	0.1358	0.0497	0.0063	1.1	(1.04, 1.26)
Previous births	0.1333	0.5137	0.7953	1.1	(0.42, 3.13)
No use of alcohol	−1.2684	0.8244	0.1239	0.3	(0.06, 1.41)
Bacterial vaginosis	−1.6383	0.7419	0.0272	0.2	(0.05, 0.83)

Table 5. Multivariate Logistic Regression Model for PLBW Using Primiparous Cases and Controls

Variable	Parameter Estimate	Standard Error	Probability Chi-Square	Odds Ratio	95% Confidence Levels
Extent 3:60	2.0701	0.8439	0.0142	7.926	(1.52, 41.4)
Black	−0.8458	0.7179	0.2388	0.429	(0.11, 1.76)
Age	0.1150	0.0673	0.0875	1.122	(0.98, 1.28)
No use of alcohol	−1.7890	1.5055	0.2347	0.167	(0.01, 3.20)
Tobacco use	0.2098	0.9353	0.8225	1.233	(0.20, 7.71)
Bacterial vaginosis	−2.3450	1.4790	0.1128	0.096	(0.01, 1.74)
History of bacteriuria	−1.9030	0.9307	0.0409	0.149	(0.02, 0.92)

relationship between periodontal status and low birth weight. This indicates that the relationship is reasonably stable. Additionally, interaction effects among the variables in the model were tested and none were significant. This indicates that the other factors in the model do not act as effect modifiers of the periodontal status and low birth weight association. A similar logistic model was developed for the primiparous cases and controls, Table 5. As in the previous model, the Extent 3:60 variable was chosen as the periodontal disease predictor variable found to be significantly associated with PLBW ($P = 0.014$), with an adjusted odds ratio of 7.9 and a 95% confidence interval of 6.27–9.58, adjusting for all other variables in the model. As before, age and race were included as control variables. Neither alcohol nor tobacco use were significant risk factors in this model due to the small numbers of subjects in this subgroup. Current bacterial vaginosis was not statistically significantly protective in this group ($P = 0.11$), but was retained since there was a trend which was significant in the overall model. A large number of other risk factors were permitted to enter into the model, however, only a history of cystitis remained in the final model at $P = 0.04$. This condition appeared protective with an odds ratio of 0.149, consistent with the use of systemic antibiotics that were prescribed to treat mothers with that condition.

Other logistic models were developed for these primiparous mothers using other periodontal indicator variables. Substituting the Extent 3 scores for the Extent 3:60 score also was significant in the model ($P = 0.04$, odds ratio = 50.4); however, the wide confidence interval did not give us much confidence in the odds ratio. Models using severity 2 scores approached statistical significance at $P = 0.056$ and produced a similar odds ratio of 6.2. Even mean CAL scores approached significance at $P = 0.06$ with an odds ratio of 3.5. Thus, considering the relatively small number of patients in this group, this finding provides further confirmation that periodontal disease is significantly associated with PLBW and that risk appears to be independent of other known human risk factors in this group.

DISCUSSION

Some discussion regarding the two design aspects of this study is warranted. The first aspect involves the recruitment of subjects. The intent was to conduct a case-control study with cases being mothers who experienced preterm PROM or preterm labor associated with low birth weights ($< 2{,}500$ g) and controls being mothers without PTL or preterm PROM who had infants weighing $\geq 2{,}500$ g. These cases and controls were to come from the same pool of potential subjects—the mothers seen at the UNC Prenatal Care Clinic who later delivered at UNC Hospitals. About 10% of these women were asked during a clinic visit to participate in the study once their babies were born, and the remainder were asked after their babies were born. The intent was to enroll all cases that occurred in the prenatal clinic pool with a comparable number of controls. Thus, cases and controls actually were determined at the time of birth, but about 10% of the study group previously had agreed to participate.

The second aspect involved the measurement of the exposure of interest—periodontal status. In 10% of the mothers, periodontal conditions were determined during a clinic visit; in the remainder, periodontal conditions were determined within 3 days after the birth of their children. A case-control design normally assumes that exposures are determined historically through interview or

records and that the exposures occurred prior to the outcome. As mentioned earlier, a periodontal examination conducted at a point in time is a measure of the periodontal experience of the individual; a single examination usually cannot determine whether the diseased sites are active at that time. Thus, a periodontal examination conducted on mothers within 3 days of their child's birth is a measurement of prior disease experience and provides the same information as any single exam conducted during the pregnancy. The advantage in this study is that all periodontal exams, whether conducted during pregnancy or after the birth, were done in a standardized manner with trained examiners. Although the periodontal disease measured in this study occurred prior to the birth, we cannot, however, determine whether the disease actually was in an active cycle during the pregnancy. This type of classification error would tend to bias the results toward the null. Thus, the data from this study have the characteristics of a case-control design: the subjects were drawn from the same pool; their inclusion in the study was based on the outcome of interest; and the exposures occurred prior to the outcome.

The causes of prematurity are multiple, as are the variables that are associated with this disease. This case-control investigation illustrates a strong association between periodontal disease and low birth weight, even after controlling for multiple contributing factors. This study does not elucidate whether the association is causal in nature; however, there are several lines of biochemical, immunologic, and histologic evidence which support the hypothesis that periodontal disease is more than an association, but is contributing to low birth weight. Thus, the potential mechanisms that may explain the relationship between periodontal disease and PLBW bear comment.

Certainly, periodontal infection can serve as a chronic reservoir of lipopolysaccharide which could target the placental membranes via the bloodstream. LPS has been shown to elicit IL-1β and PGE_2 production by the chorioamnionic and trophoblastic cells, a process often associated with preterm parturition. Alternatively, inflammatory mediators such as PGE_2 and TNF-α may be produced locally within the periodontium and, due to the high vascularity of this organ, act as a potential systemic source of fetotoxic cytokines. For example, PGE_2 can reach concentrations of 1 to 3 μmol in the inflamed periodontium, which is approximately the same mass as a pancreas that has tissue concentration of insulin of about 2 μmol. Furthermore, increased serum TNF-α levels have recently been found to be associated with the extent of disease progression in periodontitis patients who are undergoing active attachment loss.[24] These findings suggest that perhaps the infected periodontium can represent an endocrine-like source of potentially deleterious cytokines and lipid mediators. These LPS and cytokine-dependent mechanisms have been suggested by others as plausible explanations for the observed associations between oral infections and other systemic inflammatory conditions, such as vasculitis, atherosclerosis, and thromboembolic phenomena.[25] It is also possible that there is an unknown genetic or environmental confounder, that is, an underlying condition that places a patient at risk for both periodontal disease and PLBW. Certain diagnostic subcategories of periodontal patients have been described as having an up-regulated monocytic response to LPS that results in a 3- to 5- fold elevation of inflammatory mediator secretion (see 26 and 27 for review). Thus, an underlying hyper-responsive inflammatory trait may place an individual at risk for both more severe periodontitis and PLBW.

The possibility that the presence of periodontal infection may also render the patient more susceptible to subclinical bacterial vaginosis cannot be ruled out in this investigation. It has been known for many years that LPS extracted from oral organisms has distinctly different structural and biological activities than LPS extracted from enteric organisms. A recent report by Darveau et al.[28] indicated that exposure to oral LPS down-regulates E-selectin expression on endothelial cells and thereby prevents the normal leukocytic margination and diapedesis which would occur in response to a secondary enteric LPS challenge. This raises the possibility that systemic challenge with oral LPS may inhibit normal neutrophil clearance of enteric organisms that may permit a selective overgrowth or invasion of Gram-negative organisms within the genitourinary tract. Thus, oral infection that results in systemic LPS challenge may predispose or exacerbate any existent enteric challenge at a distal site.

The significance of the association between periodontal disease and PLBW warrants further investigation and reopens new possibilities for an old concept—that of focal infection. In 1931 Galloway[29] first suggested that periodontal disease, a Gram-negative anaerobic infection of the periodontium, may "provide sufficient infectious microbial challenge" to have "potentially harmful effects on the pregnant mother and developing fetus." Thus, our interest in the potential association between these highly prevalent, chronic oral infections and PLBW is not unprecedented. However, our data would indicate that the magnitude of the potentially deleterious effect of periodontal infection on the fetal-placental unit has not been fully appreciated. The periodontium has a surface area that approximates the ventral surface of the human forearm. Logically, if the entire forearm were inflamed with gross suppuration and radiographic evidence of osteomyelitis, one would not be surprised if there were systemic sequelae. Indeed, it now appears clear that the oral cavity is not an immunoprivileged site and that the potential systemic ramifications of this common anaerobic infection should be reconsidered.

The limited scope of this case-control study does not

enable broad generalizations regarding the potential health care impact of these findings. Caution must be exercised in interpreting the applicability of the current data until these findings can be confirmed by larger, prospective multicenter investigations. Nonetheless, it is tempting to use the preliminary findings to generate an estimate of the potential magnitude of the impact of periodontal disease on PLBW (adjusted for the presence of other known obstetric risk factors, excluding from the denominator LBW due to twins, fetal anomalies, pre-eclampsia, and other maternal conditions). Using the finding of an odds ratio of 7.5 combined with a generally estimated incidence of PLBW at approximately 10% of all births, the risk difference (risk attributable to periodontal infection) can be computed to be 18.2%, using the methods and assumptions described by Kleinbaum and colleagues.[30] Based on these data, 18.2% of the approximately 250,000 PLBW which occur annually may be attributable to periodontal infection. Theoretically, the elimination of periodontal infection in pregnant women could result in a reduction of approximately 45,500 PLBW births a year and a concomitant decrease in intensive care unit costs of almost $1 billion. Considering the fact that periodontal infections are both preventable and readily treated, these findings provide new opportunities for intervention strategies to reduce the incidence of PLBW.

In summary, these data provide new evidence that periodontal disease in pregnant women may be a significant risk factor for preterm low birth weight. The effect in this study was of significant magnitude, contributing to more cases of PLBW than either smoking or alcohol use. Estimates suggest that 18.2% of all PLBW cases may be attributable to periodontal disease and that periodontitis represents a previously unrecognized and clinically important risk factor for preterm low birth weight, which occurs as a sequelae to premature rupture of membranes or preterm labor at less than 36 completed weeks of gestation.

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REFERENCES

1. U.S. Department of Health and Human Services. Center for Research for Mothers and Children. *PHS-NIH Progress Report.* Washington, DC: U.S. Department of Health and Human Services; 1984.
2. McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. *N Engl J Med* 1985;312:82–90.
3. Williams RL, Chen PM. Identifying the sources of the recent decline in perinatal mortality rates in California. *N Engl J Med* 1982;306: 207–214.
4. Committee to Study the Prevention of Low Birthweight, Division of Health Promotion and Disease Prevention, Institute of Medicine. *Preventing Low Birthweight.* Washington, DC: National Academy Press; 1985.
5. National Center for Health Statistics. *Health, United States, 1982.* Washington, DC: U.S. Government Printing Office; December 1982. DHHS publication (PHS) 83-1232.
6. Gibbs RS, Romero R, Hillier SL, Eschenbach DA, Sweet RL. A review of premature birth and subclinical infections. *Am J Obstet Gynecol* 1992;166:1515–1528.
7. Evaldson G, Lagrelius A, Winiarski J. Premature rupture of the membranes. *Acta Obstet Gynecol Scand* 1980;59:385–391.
8. Taffel S. *Prenatal Care, United States, 1968–1975.* Hyattsville, MD: National Center for Health Statistics; 1978. DHEW publication (PHS) 78–1911.
9. Gortmaker SL. The effects of prenatal care on the health of the newborn. *Am J Public Health* 1978;69:653–660.
10. Patrick MJ. Influence of maternal renal infection on the foetus and infant. *Arch Dis Child* 1967;42:208–213.
11. Niswander KR, Gordon M. *The Women and Their Pregnancies. The Collaborative Perinatal Study of the National Institute of Neurological Diseases and Strokes.* Philadelphia: W.B. Saunders; 1972:252–256.
12. Moller M, Thomsen AC, Borch K, Dinesen K, Zdravkovic M. Rupture of fetal membranes and premature delivery associated with group B streptococci in urine of pregnant women. *Lancet* 1984;II: 69–70.
13. White CP, Wilkins EGL, Roberts C, Davidson DC. Premature delivery and group B streptococcal bacteriuria. *Lancet* 1984;II:586.
14. Minkoff H, Grunebaum AN, Schwartz RH, et al. Risk factors for prematurity and premature rupture of the membranes: A prospective study of the vaginal flora in pregnancy. *Am J Obstet Gynecol* 1984;150:965–972.
15. McDonald HM, O'Loughlin JA, Jolley P, Vigneswaran P, McDonald PJ. Vaginal infections and preterm labor. *Br J Obstet Gynecol* 1991;98:427–435.
16. Mueller-Heubach E, Rubenstein DN, Schwarz SS. Histological chorioamnionitis and preterm delivery in different patient populations. *Obstet Gynecol* 1990;75:622–626.
17. Hillier SL, Martius J, Krohn MJ, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamnionic infection and chorioamnionitis in prematurity. *N Engl J Med* 1988;319:972–978.
18. Romero R, Hobbins JC, Mitchell MD. Endotoxin stimulates prostaglandin E_2 production by human amnion. *Obstet Gynecol* 1988;71: 227–228.
19. Romero R, Mazor M, Wu YK, Avila C, Oyarzun E, Mitchell MD. Bacterial endotoxin and tumor necrosis factor stimulate prostaglandin production by human decidua. *Prostaglandins Leukot Essent Fatty Acids* 1989;37:183–185.
20. Collins JG, Windley HW III, Arnold RR, Offenbacher S. Effects of a *Porphyromonas gingivalis* infection on inflammatory mediator response and pregnancy outcome in the hamster. *Infect Immun* 1994;62:4356–4361.
21. Collins JG, Kirtland BC, Arnold RR, Offenbacher S. Experimental periodontitis retards hamster fetal growth. *J Dent Res* 1995;74(Spec. Issue):158(Abstr. 1171).
22. Beck JD. Methods of assessing risk for periodontitis and developing multifactorial models. *J Periodontol* 1994;65:316–323.
23. Carlos J, Wolfe M, Kingman A. The extent and severity index: A simple method for use in epidemiologic studies of periodontal disease. *J Clin Periodontol* 1986;13:500–504.
24. Moss M, Beck J, Genco R, Salvi G, Offenbacher S. Progressing periodontitis is associated with increased serum tumor necrosis factor alpha (TNFα). *J Dent Res* 1995;74(Spec. Issue):158(Abstr. 1172).
25. Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. *J Periodontol* 1996;67(suppl): 1123–1137.
26. Offenbacher S, Heasman PA, Collins JG. Modulation of host PGE_2 secretion as a determinant of periodontal disease expression. *J Periodontol* 1993;64:432–444.
27. Offenbacher S, Collins JG, Yalda B, Haradon G. Role of Prosta-

glandins in High Risk Periodontitis Patients. In: Genco R, Hamada S, Lehner T, McGhee J, Mergenhagen S, eds. *Molecular Pathogenesis of Periodontal Diseases.* Washington, DC: ASM Press; 1994: 203–214.

28. Darveau RP, Cunningham MD, Bailey T, et al. Ability of bacteria associated with chronic inflammatory disease to stimulate E-Selectin expression and promote neutrophil adhesion. *Infect Immun* 1995;63: 1311–1317.
29. Galloway CE. Focal infection. *Am J Surg* 1931;14:643–645.
30. Kleinbaum DG, Kupper LL, Morgenstern H. *Epidemiologic Research: Principles and Quantitative Methods.* Belmont, CA: Lifetime Learning;1982:144–145.

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